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PRINCIPAL INVESTIGATOR: Molly A. Thoreson, Ph.D.
Albert B. Reynolds, Ph.D.

CONTRACTING ORGANIZATION:

Vanderbilt University Medical Center
Nashville, Tennessee 37232-2103

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6. AUTHOR(S)
Molly A. Thoreson, Ph.D.
Albert B. Reynolds, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Vanderbilt University Medical Center
Nashville, Tennessee 37232-2103

E-Mail: molly.thoreson@amgen.com

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13. ABSTRACT *(Maximum 200 Words)*

The goal of our research is to understand the way in which p120-catenin regulates the function of the cell adhesion molecule E-cadherin. E-cadherin is necessary for inhibiting metastasis of human cancer cells. Previously, we uncoupled p120 from E-cadherin through small juxtamembrane domain substitutions on E-cadherin. This resulted in a loss of strong adhesion and lack of tight colony formation in cells in which p120 and E-cadherin were uncoupled. In this reporting period, we have created a tool to investigate the role of p120 in cadherin clustering and cadherin-mediated signal transduction. We purified a dimerized form of the E-cadherin extracellular domain, and showed that this protein facilitates calcium-dependent homotypic interactions. In addition, we have described a p120-deficient cell line that requires p120 transfection for normal cadherin function. Our data suggest a crucial role for p120 in E-cadherin function and stability, and give insight into possible ways to prevent or inhibit tumor metastasis.

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INTRODUCTION

The adhesion of cells to one another is a characteristic of epithelial cells which is lost during the transition from a solid to a metastatic tumor. E-cadherin is the major cell adhesion molecule found in epithelial cells, and the loss of this molecule is involved in the progression of a tumor toward metastasis. Recent evidence suggests that defects both in the cadherin itself, and in the cadherin's intracellular binding partners can encourage the transition to metastasis. Our researched is focused on understanding this process by studying the interaction of E-cadherin with p120, a cadherin binding partner originally discovered in our lab. To dissect the influence of p120 on E-cadherin function, we will use cultured human cancer cells to uncouple p120 from E-cadherin *in vivo* and examine effects on the cadherin related to cell adhesion and invasion. We expect these experiments to reveal a better understanding of the epithelial to mesynchymal transition which occurs as a loss of cadherin function, and give us a greater understanding of possible drug targets for the inhibition of tumor cell metastasis

BODY

Training: I have spent the past two years in Al Reynold's lab in the new Cancer Biology Department which is under the leadership of Dr. Lynn Matrisian. I have remained involved with the Cancer Biology Student Association, a student – initiated and run group which has been detrimental to communication between graduate students in my department. I have also been involved with the training of new and rotating graduate students in our lab, and have lectured in the Cancer Biology Department course entitled "Experimental Methods in Cancer Biology."

Research: The statement of work for this grant is included below for reference. We have completed Tasks 1, 2, and 3 and some of these results are published in the Journal of Cell Biology, 2000 (v.148, p 189-202), which was submitted with last year's annual summary. For task 1, I subcloned p120-uncoupled E-cadherin mutants into an expression vector and transfected them to obtain stable cell lines. Cells were screened by immunofluorescence and western blotting for E-cadherin, p120, and several other catenins, and I demonstrated that p120 is stable even if not bound to cadherins, and it accumulated in the cytoplasm.

In Task 2, I characterized the cell lines obtained by performing co-immunoprecipitations of p120 with the E-cadherin mutants. I found that p120 does not bind to these E-cadherin mutants although α- and β- catenin bind very well to both wild-type and mutant E-cadherin. Subcellular fractionation suggested that over 90% of p120 associates with E-cadherin, demonstrating a high stoichiometric interaction. Phosphatase treatment of cells confirmed that p120 is phosphorylated when bound to cadherins, but not phosphorylated while cytoplasmic.

Task 3 was completed by performing several assays for cadherin function. Cells containing mutant E-cadherin had loose junctions and were unable to form tight colonies on tissue culture plates. Actin staining revealed disorganized actin filaments in cells containing mutant E-cadherin, and circumferential actin cables were absent from mutant cells, even though cadherins were able to concentrate at junctions. Aggregation assays

revealed a selective inability of the mutant E-cadherin to promote strong adhesion. These observations suggest a defect in cadherin clustering due to lack of p120 binding.

We have been unable to complete Task 4 because of problems encountered with a lack of stable expression in the cell lines generated. We are making considerable progress in utilizing a retroviral vector (LZRS-pBMN – [GFP or Neo]) to express E-cadherin. These cells have been generated and appear to stably express proteins for a long time period, however the time needed to complete the *in vivo* experiments is now beyond the scope of this grant. Instead, I completed my research in the lab by investigating the involvement of p120 in cadherin clustering. To assess differences in the clustering ability of wild-type versus p120-uncoupled E-cadherin, I purified "Ecad-Fc", a dimerized form of the E-cadherin extracellular domain (obtained from Berry Gumbiner). I showed that the purified protein facilitates calcium-dependent homophilic interactions between Ecad-Fc coated beads, and facilitates interaction of Ecad-Fc coated beads with the E-cadherin on the surface of cells. These preliminary results will be followed up by others in the lab to assess signaling events resulting from the application of Ecad-Fc to cells, and to determine the specific role of p120 in these signaling events. In addition, we have found the first human cancer cell line known to be p120-deficient, called SW48. These cells have an impaired cadherin adhesion system, which can be restored by transfection of the cells with p120. These experiments suggest that p120 is necessary for normal cadherin function in human cells, and raise the possibility that cadherin function can be downregulated due to a loss of p120.

As a final note, I performed my doctoral dissertation defense during this grant period. This pre-doctoral grant was converted to a post-doctoral grant as of the dissertation defense date, April 1, 2002 with permission from the Department of Defense granting officials. I served as a post-doctoral fellow until June 1, 2002, and then accepted employment with Amgen, Inc. as an Oncology Medical Liaison, where I will be setting up clinical trials with Amgen's oncology drugs, and participating in oncology medical education for physicians, nurses, and pharmacists. Salary payments from this Army grant ceased effective June 1, 2002. This grant will be closed in September 2002 after my participation in the required Era of Hope DOD meeting in Orlando, and a final report will be submitted at that time.

STATEMENT OF WORK

Task 1: To generate the MDA231 model system for uncoupling E-cadherin and p120 function (months 1-12).

A. Subclone E-cadherin AAA mutants from yeast pGad vector to pLKpac1 expression vector.

B. Transfect E-cadherin mutants into MDA231 cells and establish stable cell lines expressing these mutants.

C. Screen cell lines for E-cadherin expression by immunofluorescence, and western blotting.

Task 2: To characterize stably transfected cell lines and identify minimal E-cadherin JMD mutants that effectively uncouple E-cadherin from p120 (months 6-18).

A. Characterize binding of wild-type and mutant E-cadherin to p120 and α- and β-catenin by co-immunoprecipitation.

B. Determine final mapping of p120 binding site on E-cadherin's JMD.

C. Perform subcellular fractionation of transfected cells to address p120/E-cadherin stiochiometry.

D. Phosphatase-treat p120 immunoprecipitates and blot for p-tyr, ser and thr to asses p120 phosphorylation state when bound to/uncoupled from E-cadherin.

Task 3: To determine the impact of uncoupling p120 and E-cadherin on adhesion, motility, and invasion by performing functional assays *in vitro*. (months 12-36).

A. Perform aggregation assays using established protocols for cadherin function.

B. Examine motility using an in vitro wound healing assay.

C. Perform invasion assays in soft agar.

Task 4: To analyze the effects of uncoupling p120 and E-cadherin on metastasis utilizing a mouse model for metastasis of breast cancer to bone (months 24-36)

A. Inject 24 female Balb/c-nu/nu (nude) mice with MDA231 cells transfected with vector alone, wild-type E-cadherin, or p120-uncoupled E-cadherin mutants.

B. Assess tumor size (radiographically) and number in animals.

C. Test ability of tumors to metastasis to bone using histological examination.

D. Measure growth rate and tumorigenicity of cells by injection into the mammary fat pad of nude mice.

KEY RESEARCH ACCOMPLISHMENTS

- Showed that p120 localizes to the cytoplasm in cadherin deficient cells, where it is stable but unable to be serine phosphorylated, and demonstrated that cadherin expression is necessary and sufficient for p120 localization to cell junctions.
- Uncoupled p120 from E-cadherin through mutation in the juxtamembrane domain of E-cadherin and made cell lines expressing these mutants which showed that p120 is necessary for strong adhesion and possibly cadherin clustering.
- Purified a dimerized E-cadherin extracellular domain construct and showed that it facilitates homophilic adhesion.
- Described a p120-deficient cell line in which the cadherin adhesion system is only functional after transfection with wild-type p120.

REPORTABLE OUTCOMES

- Publishing review article on the effects of p120 loss in human tumors:
  Thoreson MA and Reynolds AB. Altered Expression of the Catenin p120 in Human Cancer: Implications for Tumor Progression. *Differentiation*, accepted.
- Publishing recent findings in the Journal of Cell Biology:
  Ireton RC, Davis MA, van Hengel J, Mariner DJ, Barnes K, **Thoreson MA**, Anastasiadis PZ, Matrisian L, Bundy LM, Sealy L, Gilbert B, van Roy F, Reynolds AB. (2002) A Novel Role for p120 Catenin in E-cadherin Activation. *Journal of Cell Biology*, accepted.
- Published findings in the Journal of Cell Biology:
  Thoreson MA, Anastasiadis PZ, Daniel JM, Ireton RC, Wheelock MJ, Johnson KR, Hummingbird DK, and Reynolds AB. (2000) Selective Uncoupling of p120 ctn from E-cadherin disrupts Strong Adhesion. *Journal of Cell Biology*, **148**:189-202.

- Received Ph.D.from Cancer Biology Department, Vanderbilt University, May, 2002
- Received short-term post-doctoral position in Al Reynold's lab immediately after graduation
- Received employment at Amgen, Inc. as an Oncology Medical Liaison

CONCLUSIONS

Through this research, we have made advancements in understanding p120 and its effects on E-cadherin, cell adhesion, and signaling. We established a high stoichiometry between p120 and E-cadherin, and demonstrated that unlike the other catenins, p120 can accumulate to high levels in the cytoplasm where it is likely involved in signal transduction. We have shown that the binding of p120 to E-cadherin is crucial for E-cadherin's ability to mediate strong cell-cell adhesion. Currently, we are investigating the mechanism of this regulation, through work with a dimerized cadherin extracellular domain protein. Finally, our data reveals the possibility that p120 loss in human tumors precedes, and may even cause, E-cadherin loss. These observations have lead to a better understanding of the role of p120 in cell adhesion and signaling, and point toward p120 as a possible drug target for the prevention or inhibition of tumor cell metastasis.

REFERENCES

None.

APPENDICES

None.